

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Cadm3tm1Lex/Mmucd **MMRRC: 032170-UCD**

Protocol:

Reagent/Constituent	Volume (μL)
Water	5.6
GoTaq® G2 Colorless Master Mix, 2X	7.5
Primer 1. (stock concentration is 20μM)	0.45
Primer 2. (stock concentration is 20μM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME	
15	

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- The mutant PCR is a general LacZ PCR. The wild type is specific for this strain.

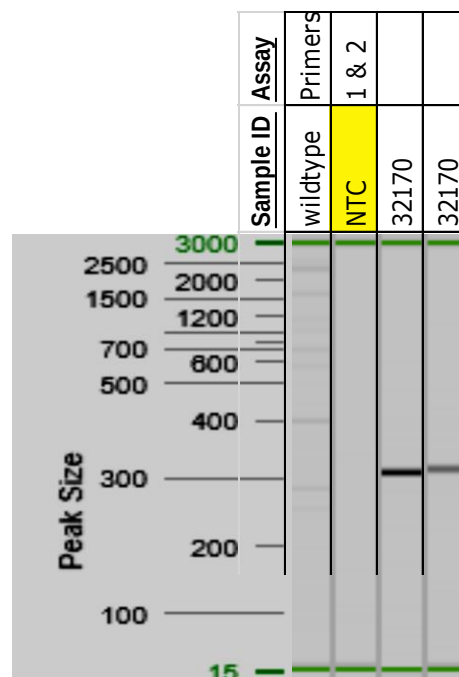
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00 (↑20sec/cycle)	

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5%	V: 90
1. DNA033-10	CCCGAGATCCACATCCCTGCTGC	Estimated Running Time: 90 min.	
2. Neo3a	GCAGCGCATCGCCTTCTATC	Primer Combination	Band (bp)
3. DNA033-5	GCGCCGCCCTCCCTCAC	1 & 2	318
4. DNA033-2	CCCCGTCACCACTCCCTCCAC	2 & 4	339
			Genotype
			mutant
			wildtype





Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ225	Date of Submission:	5/6/04
Lexicon Contract Name:	DNA033	Mutation Type:	X Standard Knock out
LexVision Name:	MEM633N1		☐ Conditional
Reference accessions:	ENSMUST00000005470	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) __58_____
 X Target Vector DNA __pKOS.DNA033.58 TV_____
 X Targeted ES Cell DNA __1A10_____
 X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	13/14	21/22
Restriction Enzyme for Genomic Digest:	Spe I	Nhe I
Predicted Wild-type Band (kb):	10.8	13.5
Predicted Mutant Band (kb):	8.0	18.7
Probe Size:	418	400

PCR Strategies:

For standard knockouts, give wildtype and mutant-specific strategies

For conditionals, give 5' loxP and cre-excision strategies

Wild type-specific (absent in targeted allele)		Mutation-specific product (absent in wt)	
5' Primer Name:	DNA033-2	5' Primer Name:	neo3a
3' Primer Name:	DNA033-5	3' Primer Name:	DNA033-10
Predicted Wild-type Band (bp):	339	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	318

5' loxP strategy		Distinguish Cre-excised and wt	
5' Primer Name:		5' Primer Name:	
3' Primer Name:		3' Primer Name:	
Predicted Wild-type Band (bp):		Predicted Wild-type Band (bp):	
Predicted mutant band (bp)		Predicted mutant band (bp)	

Primer sequences:**Southern probes**

DNA033-13 5' – ATTAAGGACAGGCAGAAGGAGACC
DNA033-14 5' – TGTAAGACTGGGAGGAGACTGGAC
DNA033-21 5' – AAGGTAGTCCAGGAGCATAACAAA
DNA033-22 5' - AGGGCCAAGGCACAGGAGGTA

PCR Genotyping

DNA033-2 5' - CCCCCGTCACCACCCCTCCAC
DNA033-5 5' - GCGCCGCCCCCTCCCTCAC
DNA033-10 5' - CCCGAGATCCACATCCCTGCTGC
Neo3a 5' - GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

ATGGGGGGCCCTTCCGCCCTGCCCTGCTCCTGCTCCTCGCCTGCTCCTGGGCGCCCGGGCGGGGGCCAATCTTTCCAGG
ACGGTGAGTGAGGGAGGGGGCGGCGCCAGGGGAGGTGGCCAGTGCCCCGAGGTGGGGGTTTCAGACGGGGAGCCGGG
GCAGGGAGCCTGTTGGCTTTGTTT

Genomic Locus: (the deleted sequence represents nt- 7911-8090 in the sequence below)

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Selection Cassette: LacZ/neo

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Targeted Locus:

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